Taylor & Francis
healthsciences

Effects of Subchronic Exposures to Concentrated Ambient Particles (CAPs) in Mice: VIII. Source-Related Daily Variations in In Vitro Responses to CAPs

Polina Maciejczyk and Lung Chi Chen

Department of Environmental Medicine, New York University School of Medicine, Tuxedo, New York, USA

The NYU PM Center subchronic animal inhalation study addressed the issues of composition and sources of ambient fine particulate matter (PM$_{2.5}$), as well as the relationship of these PM$_{2.5}$ characteristics to the cellular response of human bronchial epithelial cells. In this simultaneous study, we used an in vitro exposure technique to compare the daily variations of the responses of cells to fine concentrated ambient particles (CAPs) collected from a rural area upwind of New York City for the period of 9 a.m. to 3 p.m. on weekdays only, March–September, 2003. Chemical composition data for CAPs were modeled using factor analysis with varimax orthogonal rotation to determine four particle source categories contributing significant amount of mass to CAPs at Sterling Forest (Tuxedo, NY). These source categories are: (1) regional secondary sulfate characterized by high S, Si, and organic carbon (OC); (2) resuspended soil characterized by high concentrations of Ca, Fe, Al, and Si; (3) oil-fired power plants emissions of the eastern United States identified by presence of V, Ni, and Se; and (4) unknown other sources. To estimate the mass contributions of each individual source category, the CAPs mass concentration was regressed against the factor scores. Regional sulfate was the largest contributor to mass (65%), followed by soil (20%), residual oil combustion (2%), and the other sources contributing 13%. Based on an evaluation of the cellular responses to CAPs and a detailed chemical characterization of the ambient PM$_{2.5}$, we investigated the sources and individual components of ambient PM$_{2.5}$ that are responsible for the induced cellular response. Nuclear Factor kappa B (NF-κB) was selected as a monitor of cellular stress response that followed after the exposure to CAPs. The results of the NF-κB assay were found to be most highly correlated with Ni and V among the individual components, and with the residual oil combustion source category.

Source apportionment receptor models provide estimates of the contributions of various source categories to ambient air pollution at a given location at a given time; these methods are used to resolve the composition of particulate matter (PM) into components related to emission source categories. In order to distinguish among source type contributions, the measured chemical characteristics must be such that they are present in different proportions in different emission sources. It is assumed that

the changes in these proportions between source and receptor are negligible or can be approximated. These various sources are distinguishable by the source apportionment because they have distinctive tracers and correlations that are closely associated with each source profile.

The respiratory tract is an important absorption pathway as well as the major target organ for airborne pollutants. The epithelial cell serves as a barrier between the environment and the cell of the airway. In this position epithelial cells are able to modify cell responses in the airway via their production of cytokines. Our literature review revealed a multitude of in vitro studies where cells' cytokine and chemokine productions were investigated following the exposures to residual oil fly ash (ROFA), fresh diesel particles, carbon particles, and even ambient PM. In general, these past studies had limitations such as incomplete characterization of the particles, or concentrations of PM too low to yield measurable adverse effects. We used an in vitro

exposure technique to compare daily relative response of cells to concentrated fine ambient particles collected from a rural area upwind of New York City. Detailed chemical characterization of the ambient PM allowed us to investigate the source categories and individual components of ambient PM that were associated with the induced cellular response, nuclear factor kappa B (NF-κB).

NF-κB belongs to a family of transcription factors that are involved in the regulation of a large number of normal cellular processes, such as immune and inflammatory responses, cellular growth, and apoptosis. In addition, these transcription factors are persistently active in a number of disease states, including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease. The activation of NF-κB is thought to be part of a stress response as it is activated by a variety of stimuli that include growth factors, cytokines, radiation, and pollution. The NF-κB activation is indicative of the oxidative stress that eventually triggers the release of various cytokines. The cytokines produced by the bronchial epithelial cells after exposure to PM are capable of contributing to the local inflammatory response in the lung. Further, the release of these cytokines into the circulation could induce a systemic response that includes stimulation of the bone marrow to release leukocytes and platelets (Fujii et al., 2002; Goto et al., 2004). The obvious advantage of the source-apportioned approach is that it allowed us to examine the association between cell responses and categories of source-related pollutants that vary together (due to the same source type, or weather condition), rather than trying to separate the effects of individual pollutants.

METHODS

Particulate Sample Collection and Analysis

For the subchronic exposure studies, the VACES exposure system was installed at our Sterling Forest Laboratory (Tuxedo, NY) with an inlet on the second floor of the three-floor building facing south and southwest. The location of the study in relation to the U.S. Eastern Seaboard is shown on Figure 1. The overview methods of monitoring of the exposure atmosphere and ambient aerosol are discussed in a companion papers by Lippmann et al. (2005) and Maciejczyk et al. (2005). Briefly, the outdoor air particles were drawn through an inlet tube, with an insect and rain trap that extended approximately 2 m beyond the wall of the building. The composition of air and PM was measured at three locations: outside the laboratory building (referred as to Outdoor), after the cyclone inlet (Ambient), and after the diffusion dryers (concentrated ambient particles, CAPs). Measurements discussed in this article are limited to Ambient and CAPs for the period 9 a.m.–3 p.m. integrated $PM_{2.5}$ mass (inlet: Aerotec 2 cyclone, 330 L min^{-1}, 50% cutoff size of 2 μm). The Outdoor monitoring included semicontinuous elemental carbon (EC) and organic carbon (OC) measurements (model 5400, Rupprecht &

FIG. 1. Map of northeastern United States with the center of a wind rose superimposed over the location of the CAPs study at Tuxedo, NY.

Patashnick, East Greenbush, NY). Teflon filter samples were collected between March 4 and September 5, 2003, on weekdays only, 9 a.m.–3 p.m. Meteorological parameters relevant to this manuscript included wind speed and wind direction as measured on the roof of the laboratory.

All PM samples for gravimetric and elemental (via energy-dispersive x-ray fluorescence, ED-XRF) analyses were collected on Teflon filters (Gelman Teflo, 37 mm, 0.2 μm pore). Filter samples were stored at constant temperature and relative humidity ($21 \pm 0.5°C$, $40 \pm 5\%$ RH) until analyzed. Filter masses were measured on a microbalance (model MT5, Mettler-Toledo Inc., Highstown, NJ). Analyses for 34 elements (listed in Table 1) followed by nondestructive XRF (model EX-6600-AF, Jordan Valley) using five secondary fluorescers (Si, Ti, Fe, Ge, and Mo),

TABLE 1

Arithmetic mean, median, standard deviation, and percentage of data above detection limit (%det) and correlation of Ambient and CAPs concentrations (ng/m^3)

	Ambient				CAPs				
	%Det	Mean	Median	SD	%Det	Mean	Median	SD	r^a
Na	19%	92	65	92	33%	460	150	620	.804
Mg	11%	43	28	25	18%	180	99	120	.392
Al	27%	130	64	190	43%	490	330	440	.679
Si	40%	95	47	90	80%	910	650	790	.769
P	39%	98	30	140	56%	790	320	1200	.960
S	100%	1500	1000	1500	100%	12,000	8400	12,000	.963
Cl	5%	18	12	43	9%	66	33	200	.858
K	65%	29	25	22	87%	240	190	180	.858
Ca	79%	22	18	18	98%	190	140	150	.804
Sc	0%	NA	NA	NA	0%	NA	NA	NA	
Ti	42%	7	5	4	60%	40	35	33	.027
V	12%	3	2	3	38%	24	6	35	.869
Cr	17%	4	3	5	29%	16	7	14	.140
Mn	9%	3	2	5	11%	8	5	7	.325
Fe	92%	36	26	35	100%	350	300	230	.729
Co	2%	2	2	0	2%	4	4	3	.013
Ni	31%	3	1	4	62%	24	18	24	.637
Cu	16%	4	3	6	44%	21	15	24	.129
Zn	63%	8	6	8	100%	80	69	49	.776
Ga	19%	4	3	3	29%	13	7	11	.542
Ge	36%	5	3	3	42%	15	6	10	.445
As	2%	8	8	4	4%	20	18	8	.573
Se	33%	5	3	3	40%	16	6	12	.257
Br	13%	5	4	3	49%	30	24	23	.484
Rb	11%	5	4	3	17%	17	9	15	.638
Sr	3%	8	9	3	1%	20	19	3	.076
Cd	1%	14	12	3	1%	46	36	89	.261
In	1%	22	20	4	2%	72	60	36	.314
Sn	13%	17	15	9	20%	61	37	42	.146
Sb	0%	NA	NA	NA	2%	37	33	20	
Te	1%	12	11	6	6%	36	31	18	.001
I	1%	8	8	2	2%	21	20	8	−.022
Cs	2%	7	7	1	0%	NA	NA	NA	
Ba	0%	NA	NA	NA	1%	38	35	15	.053
Pb	11%	15	13	11	19%	57	28	57	.631
OC	81%	1900	1700	970	82%	15,000	15,000	7500	.793
EC	76%	180	140	120	79%	1500	1400	980	.855
Nitrate	13%	480	310	560	40%	2000	950	3000	.870

[a]Correlation coefficient r between Ambient and CAPs concentrations.

and spectral software XRF2000v3.1 (U.S. EPA and ManTech Environmental Technology, Inc.). The XRF instrument was calibrated with thin elemental layers deposited on Nuclepore film substrates (Micromatter Co., Eastsound, WA) for elements ranging from atomic number $Z = 11$ (Na) to $Z = 82$ (Pb) with deposited masses gravimetrically determined to ±5%, and dual-element polymer films (U.S. EPA and ManTech Environmental Technology, Inc.). The gain and baseline of the instrument were checked before each batch of samples using copper and tin foils. Additionally, check standards and NIST SRM 2783 were run in the middle and at the end of each batch. The filters were analyzed then by ion chromatography (IC, Dionex) with a conductivity detector to determine the concentrations of water soluble sulfate, nitrate, chloride, and phosphate.

The detection limit for each element was defined as the uncertainty of the measurements (1σ), and was determined for the XRF measurements by error propagation using the following sources of random error: calibration uncertainty (±5%), long-term system stability (±5%), uncertainty in least-squares fit, uncertainty in attenuation correction, uncertainty in interference correction, uncertainty in flow rate (±5%), and uncertainty in sample deposit area (0.1 cm^2).

Detection of NF-κB

A Biosampler (SKC, Inc., Fullerton, CA) was operated between March 25 and September 5, 2003, with the exception of April 14 and May 7. In total, 110 samples were collected. Prior to cell exposure, each Biosampler sample was diluted with cell media for a final concentration of 300 μg PM/ml.

BEAS-2B cells are epithelial cells that were isolated from normal human bronchial epithelium obtained from autopsy of a non-cancerous individual (American Type Culture Collection, Manassas, VA). These cells were infected with an adenovirus 12-SV40 virus hybrid and cloned. These immortal cells retain the ability to undergo squamous differentiation in response to serum, and are widely used to screen chemical and biological agents for ability to induce or affect differentiation, cytokine production, and gene expression, as well as in mechanistic studies (e.g., Quay et al., 1998; Sasaki & Manabe, 2004; Ito et al., 2004). In this study, we used the BEAS-2B cells rather than primary cells in order to eliminate the variation in responses that is expected when cells are cultured from a variety of donors with individual biological diversity. Primary cells can undergo only a specific number of divisions until cell death, requiring the use of a new cell colony from a different donor if the cell studies are conducted in different time frames. Since we collected PM samples on different days, the primary cells might introduce another unknown variable into our end results. Use of BEAS-2B cells eliminated that confounding factor of biodiversity, and ensured uniform and reproducible responses to pollutant exposure of a homogeneous cell population. This type of assessment is critical in the first stages of examining mechanisms underlying pollutant-induced injury.

The protocol for NF-κB detection using a Luciferase assay was described previously (Huang et al., 2001). Briefly, BEAS-2B cells stably transfected with NF-κB luciferase reporter gene are cultured in DMEM (Dulbecco's modified Eagle's medium, Bio-Whittaker) supplemented with 10% FBS (fetal bovine serum, Cambrex), 100 IU/ml penicillin (Cellgro), 100 μg/ml streptomycin (Cellgro), and 2 mM L-glutamine (Gibco). The cells were cultured at 37°C in a 5% CO$_2$ incubator. The cultures were dissociated with trypsin–ethylenediamine tetraacetic acid (Gibco) and transferred to new 35-cm^2 flasks once a week. The cells were seeded to 96-well plates at a density of about 9×10^4/well, and cultured until 95% confluence. Cells were treated with an aliquot of the PM suspension for 24 h. The negative control was medium-treated cells; the positive control was vanadyl sulfate trihydrate- (200 nM, 100 nM, 50 nM, 12.5 nM) and TPA (10 ng/ml)-treated cells. After applying the lysis reagent and luciferase assay system reagents (Promega), the cell responses in terms of NF-κB were determined with a luminometer, and are discussed in terms of the signal increase compared to the negative control.

Source Apportionment and Cell Response

With respect to the source apportionment conducted in this work, concentrations of various chemical species at the receptor (sampling) site were analyzed in order to identify major particle source categories. In total, 126 CAPs samples and 22 variables were used for modeling purposes. Only compositional data (Na, Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Pb, OC, EC, and nitrate) were used as independent variables in the factor analysis. Species with detectability less than 10%, as well as elements for which the sources are not well defined (Ga, Ge, Rb, and Sn) were excluded. Factor analysis with varimax orthogonal rotation was performed with the computer program S-Plus 2000 (version 4.6, MathSoft, Seattle, WA). The number of factors in the final solution was determined by experiments with different number of factors, with the final choice based on the evaluation of the interpretability of the resulting source profiles. In a six-source model, one factor was identified as nonsignificant ($p > .01$) by the statistical analysis of individual source coefficients. The difference between a four- and five-source model was in separation of the soil factor in a way that was not explainable; additionally, there was a very small contribution of the fifth source to the overall mass. Thus, the four-solution model was selected. To obtain the daily mass contribution from each source, the factor scores were regressed onto the gravimetric mass concentrations (Thurston & Spangler, 1985). Finally, daily sources' mass contributions were correlated with daily NF-κB levels.

Meteorological considerations, such as wind direction and speed, are useful in conjunction with source apportionment to determine where contributions might have come from by indicating the air mass transport. In this work, half-hourly wind speed and direction values were averaged for the daily sample collection hours. Calm winds (wind speed <1 m/s)

were excluded due to the ambivalent behavior of the wind vane under the calm winds. Additional assessment of the daily air mass movement was accomplished by the NOAA HYSPLIT (hybrid single-particle Lagrangian integrated trajectory) model, which was run interactively on the NOAA web site (http://www.arl.noaa.gov/ss/models.hysplit.html). This model computes simple air parcel trajectories in forward (i.e., where an air parcel went to) or backward (i.e., where an air parcel came from) directions. Meteorological data for backward trajectory modeling were downloaded from the EDAS archive (horizontal resolution of about 80 km). The 24-h backward trajectories starting at the height of 20 m and 100 m above the ground level were computed every day, producing 12 trajectories per sample. The combination of source apportionment, wind directions, and back trajectories provided information on the source categories for integrated samples and indicated the air mass origin.

RESULTS AND DISCUSSION

The elemental concentrations in Ambient and CAPs filter samples were generally highly correlated, with the exception of elements that were close to the detection limit of the XRF analysis (Table 1). Some elements' detectabilities were better for CAPs than for Ambient, since more material was collected in the former samples. However, more improvement was observed for elements determined by their primary Kα lines in the XRF method (Na through Zn), while the L and M line-detected elements are more subject to interference, and benefited less from overall higher mass. The XRF S was well correlated with sulfate determined via IC (slope = 2.71, r^2 = .90), so we excluded IC sulfate from the further analyses. Correlations of XRF P and Cl with phosphate and chloride ions by IC were poor, due to the high detection limits of the latter, and thus a very small data set was available for the comparison. Thus, phosphate and
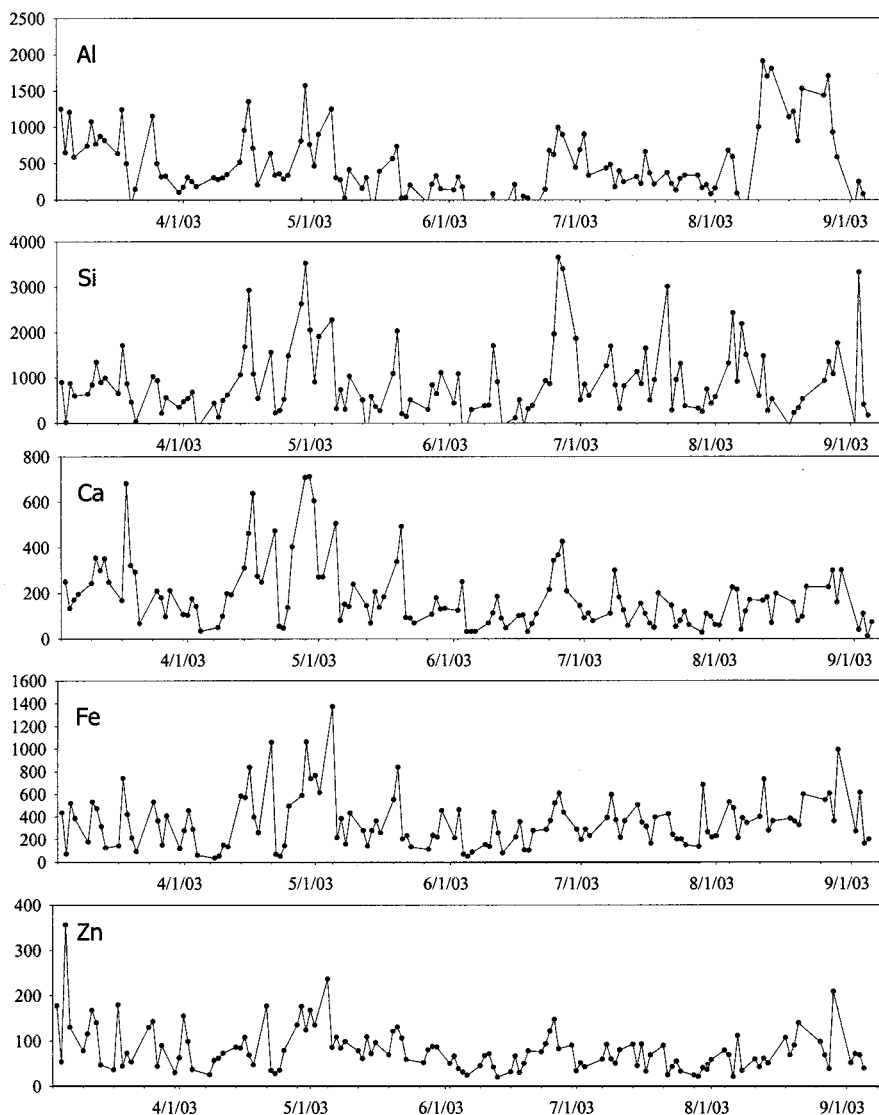

FIG. 2. Time series of selected elemental concentrations (ng/m^3) measured in CAPs. *(Continued)*
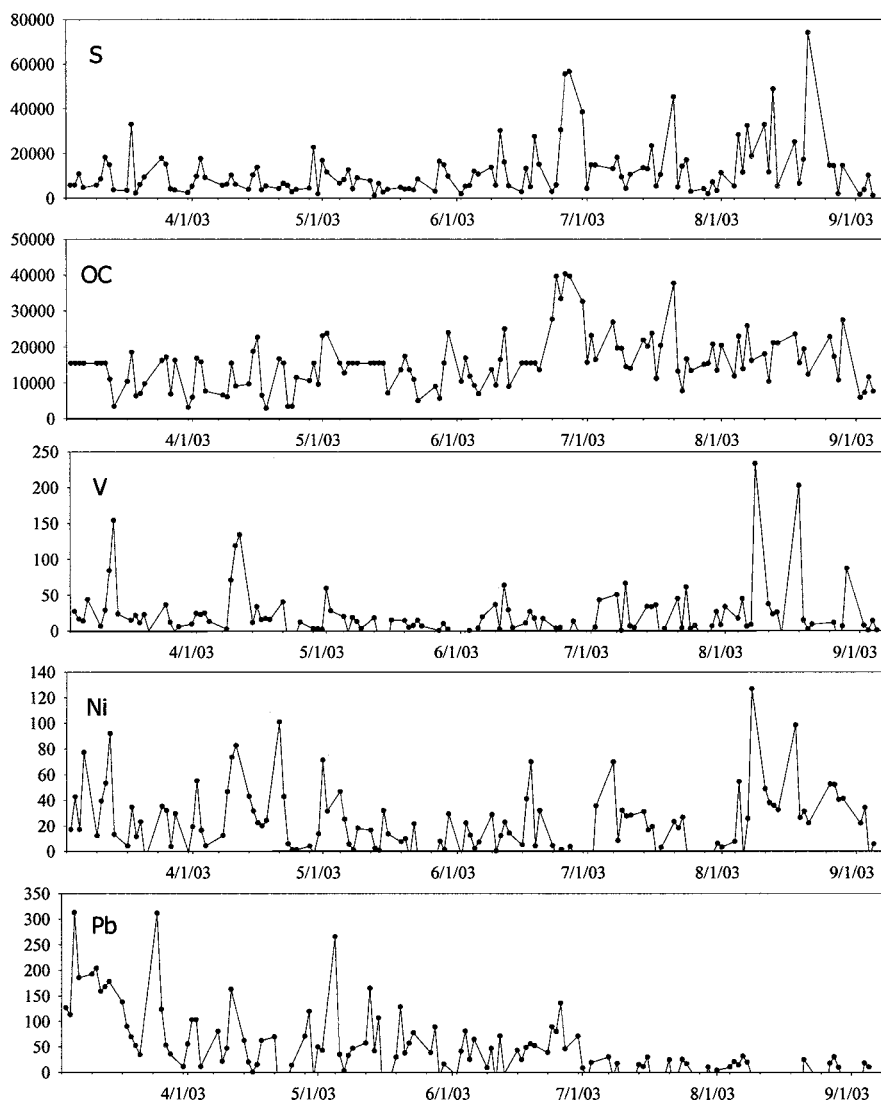
FIG. 2. (Continued)

chloride ions were also excluded from the further analyses. The arithmetic means and standard deviations of gravimetric mass concentrations for Ambient and CAPS samples were 12.6 ± 9.3 and $108.6 \pm 177.5 \ \mu g/m^3$, with the r^2 of the gravimetric mass correlation being .929 (Maciejczyk et al., 2005). The sum of all XRF-analyzed elements, EC, OC, and nitrate was on average $34.8 \pm 12.4\%$ of the CAPs mass.

During the study, the site was under the influence of air masses brought from the Midwest region of the United States under south- and northwesterly winds, and was influenced by industrial and traffic emission of the Northeast urban corridor under the south-southwest and northeast winds. The direct west and north winds were not recorded by our wind vane because the mountain ridges that surround the study site diverted winds west or east. However, our wind direction data correlated well with the wind data from a local airport located

15 miles north-northeast of the sampling site. Additionally, air mass origins for the top 5 mass contribution days from each individual source category were confirmed with 24-h backward trajectories.

In order to provide a general insight into the aerosol composition and its temporal trend, we present the time-series plots of selected chemical components measured during the daily 6-h CAPs exposure (Figure 2). These key trace elements were later used to identify the source categories from the factor analysis. Aluminum, Si, Ca, Fe, and Zn (all soil components) showed elevated levels in mid-April, and beginning of May. However, the pattern for Al and Zn differed from other soil elements in the mid-August and end-of-August period, which was an indication of other source(s) of these element. The Pb concentrations were higher at the beginning of sampling period, with several spikes up to 310 ng/m³, and then diminished significantly. Time series
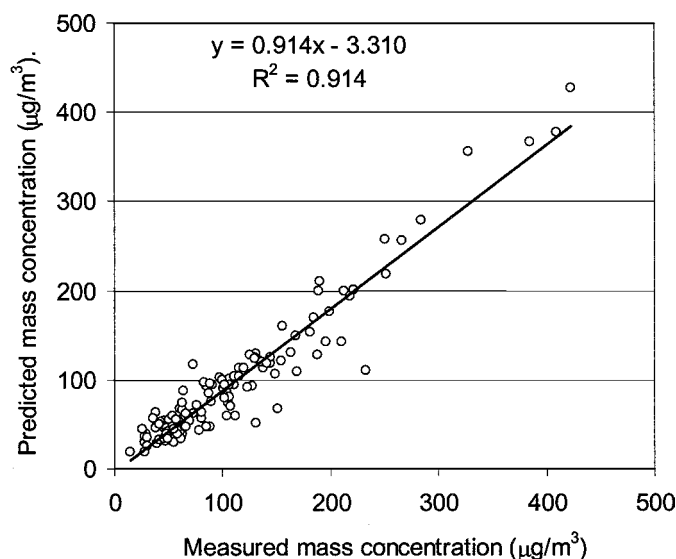
FIG. 3. Measured CAPs mass concentration versus predicted by sum of all sources' contributions.

of S and OC (as well as V and Ni) correlated well to each other, indicating that they came from the same emission sources.

As shown in Figure 3, a comparison of the daily reconstructed CAPs mass (calculated as sum of mass contributions from all sources) and measured CAPs mass concentration indicates that predicted source categories effectively reproduce the measured values and explain most of the variation in particle mass concentration. Table 2 presents the four identified source categories' loadings. Figure 4 shows time-series plots of estimated daily mass contribution from each individual source category, and in Figure 5 these daily source category contributions are paired with wind direction to form a pollution wind rose.

As shown in Table 2, the factor analysis of the CAPs compositional data identified four PM source categories on the basis of their key (highly correlated) trace elements: (1) regional secondary sulfate aerosol characterized by high S, Si, and organic carbon (OC); (2) resuspended soil characterized by high concentrations of Ca, Fe, Al, and Si; (3) oil-fired power plants emissions of the eastern United States identified by presence of V, Ni, and Se; and (4) unknown other sources.

TABLE 2
Factor analysis varimax orthogonal rotation results loadings[a] and correlation of chemical species with NF-κB response

	Secondary sulfate	Resuspended soil	Oil combustion	Other sources	r (NF-κB)[b]
Na	−0.12	0.22	0.10	0.13	0.119
Mg	0.09	0.39	−0.05	0.13	−0.002
Al	0.03	**0.62**	0.08	0.15	0.026
Si	**0.67**	**0.60**	−0.12	0.09	−0.144
P	**0.98**	−0.13	−0.10	−0.11	−0.198
S	**0.68**	0.06	0.03	0.13	−0.12
K	0.33	**0.59**	0.06	0.18	−0.075
Ca	0.12	**0.83**	−0.04	0.29	0.005
Ti	0.03	0.45	−0.13	0.30	0.021
V	0.19	−0.01	**0.75**	0.13	**0.289**
Cr	−0.04	0.23	0.08	−0.03	−0.143
Mn	0.10	**0.51**	0.09	−0.09	0.049
Fe	0.24	**0.87**	0.10	0.14	−0.076
Ni	0.05	0.17	**0.92**	0.14	**0.302**
Cu	−0.06	0.07	0.14	0.25	0.033
Zn	0.18	**0.56**	0.10	**0.46**	−0.063
Se	0.04	0.06	0.11	**0.72**	0.033
Br	0.25	0.23	−0.16	**0.86**	−0.076
Pb	0.03	0.09	−0.02	**0.80**	−0.02
OC	**0.68**	0.22	0.04	−0.03	−0.176
EC	**0.55**	0.21	0.21	0.00	−0.096
Nitrate	−0.03	0.13	0.21	**0.48**	0.031

Note. Bold values indicate key (highly correlated) elements.

[a]Loadings = correlations of each element with each source component.

[b]Correlation coefficient r between the chemical species and NF-κB response.
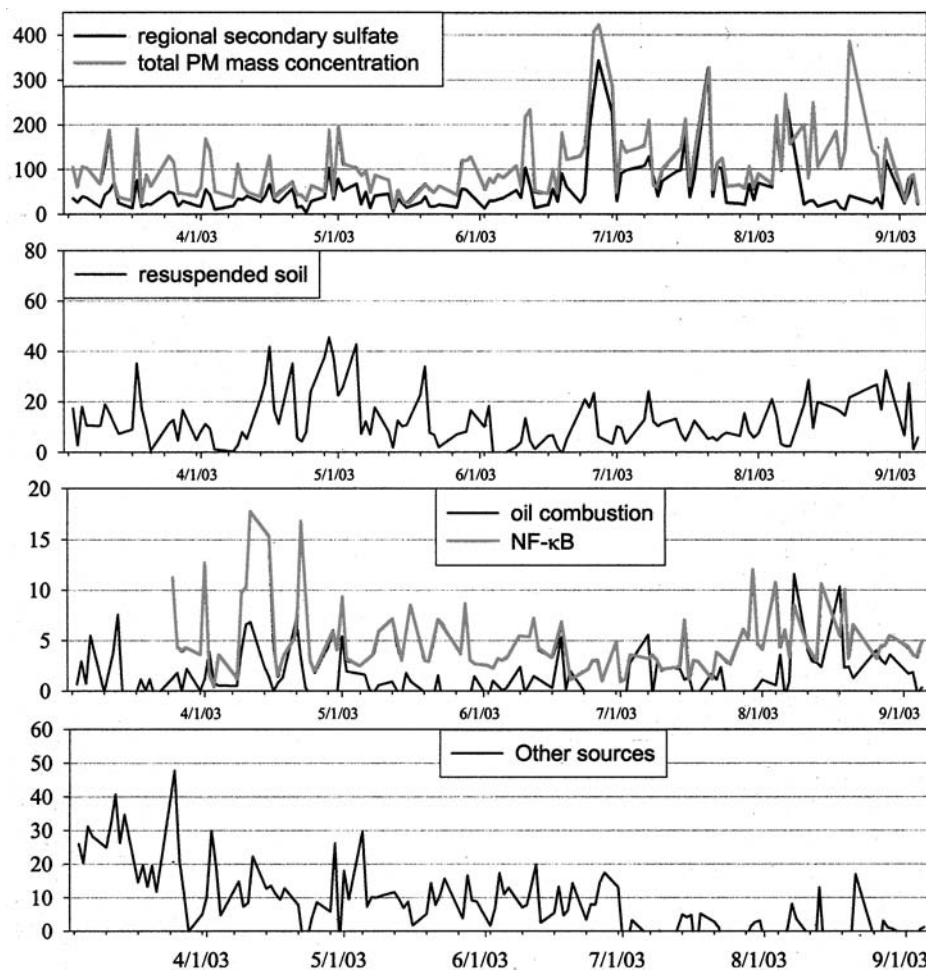
FIG. 4. Time-series plot of each source category mass contribution (μg/m^3). Additional plots are total PM mass concentration (μg/m^3) in secondary sulfate plot, and cellular NF-κB response (in units of times over the negative control) in oil combustion plot.

Regional secondary sulfate aerosol consists of the emissions from coal-fired power plants (CFPPs) of the U.S. Midwest region that reaches our sampling site under westerly winds, and was identified by enriched levels of S and Si. Selenium, commonly attributed to the coal combustion, is a better source for tracing local CFPPs (Ondov & Wexler, 1998). In fact, our factor analysis yields a small Se value in loadings (Table 2), indicating a long-range transport origin that was also confirmed by wind direction analyses. Sulfur is emitted from CFPPs as sulfur dioxide, which undergoes the photochemical oxidation and conversion to sulfate secondary aerosol. S-rich secondary aerosol shows a somewhat seasonal increase over the summer month when the photochemical activity is the highest. Indeed, within the duration of the exposure study, the top five regional sulfate contributions to mass were during the months of June, July, and the beginning of August. With several regional sulfate episodes observed, the highest S concentration in CAPs was over 75 μg/m^3. The regional aerosol was also associated with OC (correlation

coefficient $r = .68$) and EC ($r = .55$), consistent with previous findings (Song et al., 2001). This source contributed on average $65 \pm 24\%$ to overall particulate mass, and coincided well with the total mass concentrations in the Figure 4 time-series plot. The NF-κB response was not correlated ($r = -.172$) with the secondary sulfate source category, suggesting that airborne PM$_{2.5}$ from coal combustion maybe a weak indicator of cellular oxidative stress.

The resuspended soil factor is characterized by contributions of Ca, Si, Al, Fe, and Mn. No seasonal pattern was observed. As shown by the pollution rose in Figure 5, there was no prevailing wind direction for this source, as its rose was quite similar to the general wind rose recorded at the site. Local unpaved roads and trails, as well as construction and landscaping of a new state park visiting center in close proximity to the laboratory, could have contributed to these crustal elements. Additionally, the penetration of some coarse particles into the CAPs should not be excluded due to the nonsharp cutoff nature of the sampling
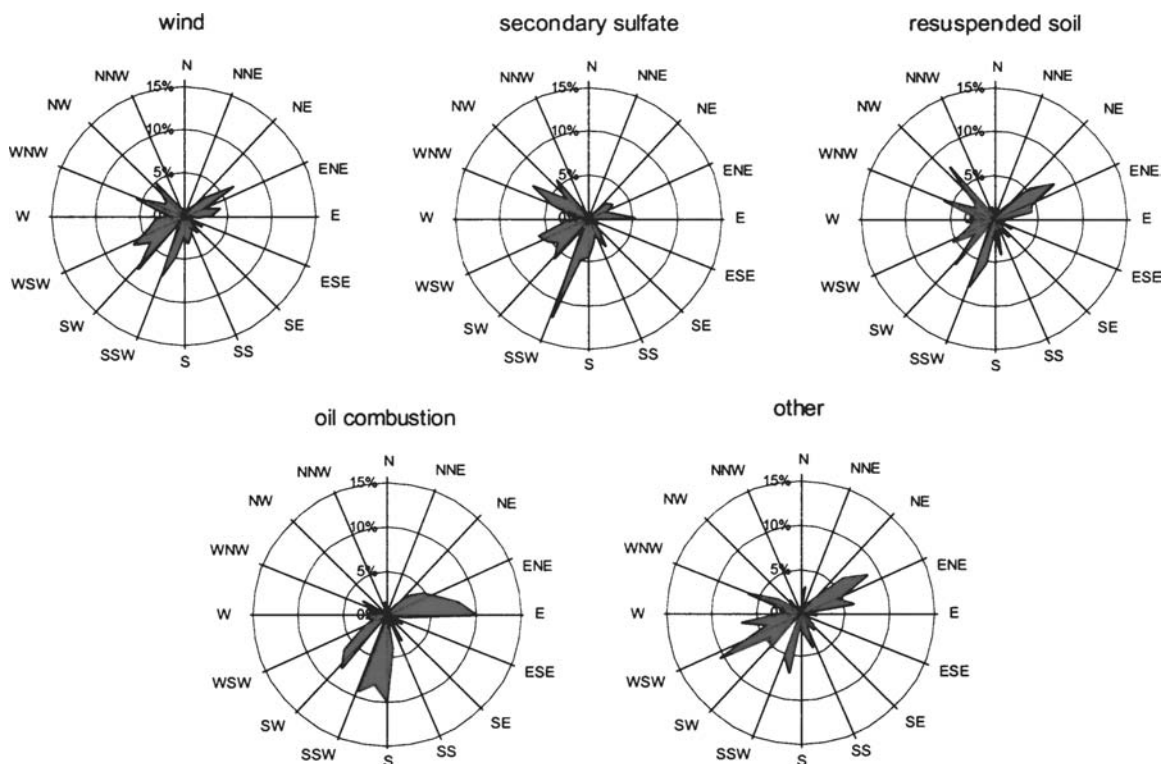
FIG. 5. Wind and pollution roses of each source category mass contribution (%).

inlet (Maciejczyk et al., 2005). Overall, the resuspended soil source contributed on average 20 ± 15% to the CAPs mass concentration.

Effluents of oil combustion by electrical utilities and industrial and large commercial boilers were identified by enriched levels of Ni and V. Typical fuel oils also contain S, Al, Na, Fe (Miller et al., 1998), As, Cu, Mn (Buerki et al., 1989), and other elements. Oil also contains Se (U.S. EPA, 2003), which was better correlated with our oil combustion source category than with secondary sulfate. Upon the emission from the stacks, the particles contain Fe, Mg, and Ni in the core, while V, a known catalyst for SO_2 to SO_3 oxidation, is associated with sulfate (Miller et al., 1998). In fact, oil-fired boilers are potentially important source of acid aerosol. Although cleaner number 2 oil is burned for residential space heating during the colder months, the residual oils numbers 4, 5, and 6 are burned throughout the year for electricity production. The south-southwest origin, shown by the pollution rose in Figure 5, is consistent with the oil burning from oil-fired power plants located along the east coast of the United States (Poirot et al., 2001; Polissar et al., 2001). Most certainly there was also an influence of four moderately sized electric utility boilers that burn both coal and oil numbers 2 and 6, about 20 miles northeast and east of the study site. The loading for Se, a tracer for fresh CFFPs emissions, indicates the possibility of some contribution of local coal combustion to this general oil combustion source category. A spike on August 8 was due to the

emissions of these local power plants brought by the east winds that occasionally occur (see Figure 5). Of particular interest, the August 18 spike in the oil combustion source may be related to the non-typical activities following the blackout of 2003, which left New York State and adjacent states without electrical power on August 14.

The NF-κB response was notably correlated with V and Ni, as shown in Table 2. These elements were correlated with oil combustion source category, as r was 0.30 and 0.29 for Ni and V, respectively, in the factor loadings shown in Table 2. Thus, not surprisingly, the NF-κB response correlated with oil combustion source category, as indicated by coefficient r in Table 3. However, it is surprising that the 2 ± 3% mass contribution that originates from this source category may be responsible for most of the cellular response. Our findings provide new and provocative evidence linking particulate air pollution from oil combustion to adverse health effects–implied by the U.S. Environmental Protection Agency (EPA) use of residual oil fly ash (ROFA) in PM toxicology studies. Our results may also help to explain why the Northeastern population exhibits greater PM-related mortality impacts than other parts of the country. Previously, the National Morbidity Mortality Air Pollution Study (NMMAPS) investigating the effect of PM10 on mortality by geographic site in 90 U.S. cities indicated that the effect is greater in the Northeast (Samet et al., 2000). Additionally, relative rates of total, cardiovascular–respiratory diseases, and other causes

TABLE 3
Correlation coefficients (r) of the cellular response NF-κB with the sources identified by factor analysis

	NF-κB	Secondary sulfate	Resuspended soil	Oil combustion	Other sources
NF-κB	1	−.172	−.020	.316	.035
Secondary sulfate		1	−.019	−.023	.088
Resuspended soil			1	.010	.035
Oil combustion				1	−.090

of mortality are larger in the Northeast and in the southern California regions (Dominici et al., 2003), where most of the oil-fired power plants in the United States are located.

The factor analysis also resolved one less well-defined source category that contributed 13% to total mass concentration. The fourth source category contained the factor with high loadings of Pb, Br, Zn, Se, and nitrate. Olmez et al. (1988) found Pb, Zn, K, Na, Cl, and sulfate to be major constituents of incinerator effluent aerosol. Antimony and Cd, good tracers of incinerator emissions, were not used in our modeling due to low detectability. The presence of Br and Zn could be an indication of the motor vehicle influence, since both elements are present in brake pads. This source has very weak wind prevalence, indicating the ubiquitous nature of the traffic source. This source category is not related to regional sulfate, soil, or oil combustion since its correlation coefficient was very small ($r < .1$, Table 3). Further identification of source contributors to this category was limited by our sampling hours (9 a.m.–3 p.m.): (1) The rush hours for the limited traffic volume in the vicinity of Tuxedo, NY, were clearly missed; and (2) other possible sources that operate beyond these hours were not sampled. Indeed, the comparison of elemental concentrations of CAPs samples with concurrent 24-h samples (data not shown) showed poor correlation for several key trace elements such as As, Pb, Ba, Sb, and EC ($r = .207$, $.331$, $.115$, $.316$, and $.393$, respectively). However, the NF-κB response showed no correlation with this source category.

SUMMARY

Source apportionment models can often be used to determine the relative contributions from individual sources within a source category to better focus control strategies. We determined that four distinct source categories contributed to the mass concentrations of CAPs, and estimated daily levels of source-apportioned PM mass concentrations for each source category. Regional sulfate was the largest contributor to mass (65%), followed by soil (20%), unknown other (13%), and oil combustion (2%) source category. These source contributions are comparable with those determined for summertime samples collected in Vermont, where 60% of mass was attributed to Midwest coal combustion, 4% to soil, and 4% to East Coast oil sources (Polissar et al., 2001).

The source-oriented evaluation of the effects of PM on cellular response was the ultimate goal of this project. Here, using the factor analysi-based source categories we established that the strongest association between the NF-κB response and the four source categories was with the oil-combustion source category.

REFERENCES

Buerki, P. R., Gaelli, B. C., and Nyffeler, U. P. 1989. Size-resolved trace metal characterization of aerosols emitted by four important source types in Switzerland. *Atmos. Environ.* 23(8):1659–1668.

Dominici, F., McDermott, A., Zeger, S. L., and Samet, J. M. 2003. National maps of the effects of particulate matter on mortality: Exploring geographical variation. *Environ. Health Perspect.* 111(1):39–44.

Fujii, T., Hayashi, S., Hogg, J. C., Mukae, H., Suwa, T., Goto, Y., Vincent, R., and van Eden, S. F. 2002. Interaction of alveolar macrophages and airway epithelial cells following exposure to particulate matter produces mediators that stimulate the bone marrow. *Am. J. Respir. Cell Mol. Biol.* 27:34–41.

Goto, Y., Hogg, J. C., Shih, C.-H., Ishii, H., Vincent, R., and van Eeden, S. F. 2004. Exposure to ambient particles accelerates monocyte release from the bone marrow in atherosclerotic rabbits. *Am. J. Physiol. Lung Cell Mol. Physiol.* 287(1):L79–85.

Huang, C., Li, J., Costa, M., Castranova, V., Vallyathan, V., Ju, G., and Shi, X. 2001. Involvement of hydrogen peroxide in nickel subsulfide-induced NFAT activation. *Cancer Res.* 61:8051–8057.

Ito, K., Hanazawa, T., Tomita, K., Barnes, P. J., and Adcock, I. M. 2004. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: Role of tyrosine nitration. *Biochem. Biophys. Res. Commun.* 315:240–245.

Lippmann, M., Gordon, T., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice: I. Introduction, objectives and experimental plan. *Inhal. Toxicol.* 17(4–5):177–187.

Maciejczyk, P., Zhong, M., Li, Q., Xiong, J., Nadziejko, C., and Chen, L. C. 2005. The design of a concentrated ambient particulate matter exposure system for biometric telemetry monitoring. *Inhal. Toxicol.* 17(4–5):189–197.

Miller, C. A., Linak, W. P., King, C., and Wendt, J. O. L. 1998. Fine particle emissions from heavy fuel oil combustion in a firetube package boiler. *Combust. Sci. Technol.* 134:477–502.

Olmez, I., Sheffield, A. E., Gordon, G. E., Houck, J. E., Pritchett, L. C., Cooper, J. A., Dzubay, T. G., and Bennett, R. L. 1988. Compositions of particles from selected sources in Philadelphia for receptor modeling applications. *J. Air Pollut Control Assoc.* 38(11): 1392–1402.

Ondov, J. M., and Wexler, A. S. 1998. Where do particulate toxins reside? An improved paradigm for the structure and dynamics of the urban Mid-Atlantic Aerosol. *Environ. Sci. Technol.* 32:2547–2555.

Poirot, R. L., Wishinski, P. R., Hopke, P. K., and Polissar, A. V. 2001. Comparative application of multiple receptor methods to identify aerosol sources in northern Vermont. *Environ. Sci. Technol.* 35:4622–4636.

Polissar, A. V., Hopke, P. K., and Poirot, R. L. 2001. Atmospheric aerosol over Vermont: Chemical composition and sources. *Environ. Sci. Technol.* 35:4606–4621.

Quay, J. L., Reed, W., Samet, J., and Devlin, R. B. 1998. Air pollution particles induce IL-6 gene expression in human airway epithelial cells via NF-κB activation. *Am. J. Respir. Cell Mol. Biol.* 19:98–106.

Samet, J. M., Zeger, S. L., Dominici, F., Curriero, F., Coursac, I., Dockery, D. W., Schwartz, J., and Zanobetti, A. L. 2000. *The national morbidity, mortality, and air pollution study. Part II: Morbidity, mortality, and air pollution in the United States.* Cambridge, MA: Health Effects Institute, research report 94.

Sasaki, K., and Manabe, H. 2004. KF19514, a phosphodiesterase 4 and 1 inhibitor, inhibits TNF-α-induced GM-CSF production by a human bronchial epithelial cell line via inhibition of PDE4. *Inflam. Res.* 53:31–37.

Song, X. H., Polissar, A. V., and Hopke, P. H. 2001. Source of fine particle composition in the Northeastern U.S. *Atmos. Environ.* 35:5277–5286.

Thurston, G. D., and Spangler, S. D. 1985. A quantitative assessment of source contributions to inhalable particle matter pollution in metropolitan Boston. *Atmos. Environ.* 19:9–25.

U.S. Environmental Protection Agency. 2003. *Report AP-42 update.* http://www.epa.gov/ttn/chief/ap42/ch01/final/c01s03.pdf.